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Review

The role of 11 β -hydroxysteroid dehydrogenase type 2 in human hypertension

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ABSTRACT

Cortisol and aldosterone have the same *in vitro* affinity for the mineralocorticoid receptor (MR), although *in vivo* only aldosterone acts as a physiologic agonist of the MR, despite circulating levels of cortisol in humans and corticosterone in rodents being three orders of magnitude higher than aldosterone levels. In mineralocorticoid target organs the enzyme 11 β -hydroxysteroid dehydrogenase type 2 (11 β HSD2) inactivates 11-hydroxy steroids, to their inactive keto-forms, thus protecting the nonselective MR from activation by glucocorticoids. The gene is highly expressed in all sodium-transporting epithelia, particularly in the kidney and colon, but also in human placenta and vascular wall. Mutations in the HSD11B2 gene cause a rare monogenic juvenile hypertensive syndrome called apparent mineralocorticoid excess (AME). In AME, compromised 11 β HSD2 enzyme activity results in activation of the MR by cortisol, causing sodium retention, hypokalaemia, and salt-dependent hypertension. Whereas mutations or inhibition of 11 β HSD2 by licorice have been clearly shown to produce a congenital or acquired syndrome of mineralocorticoid excess, the questions remaining are the extent to which subtle abnormalities in MR/11 β HSD2 mechanisms may contribute to essential hypertension. Studies in patients with essential hypertension showed a prolonged half-life of cortisol and an increased ratio of urinary cortisol to cortisone metabolites, suggesting a deficient 11 β HSD2 activity. These abnormalities may be genetically determined, as suggested by the association of a microsatellite flanking the *HSD11B2* gene with hypertension in black patients with end-stage kidney disease and with salt sensitivity of blood pressure in healthy subjects. These findings indicate that variants of the *HSD11B2* gene may contribute to the enhanced blood pressure response to salt and possibly to hypertension in humans.

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1. Introduction

In 1974 Werder et al. [1] described a case of a 3-year-old girl with short stature, polydipsia and polyuria without obvious external abnormalities (including genitalia), and who had features of mineralocorticoid hypertension with hypokalemia, metabolic alkalosis, and suppressed plasma renin and aldosterone. Gas-chromatographic analysis of her urinary steroid profile excluded hypertensive forms of congenital adrenal hyperplasia, but showed an unexpected steroid profile that the authors reported, but failed to recognise its relevance [1]. Translumbar retroperitoneal carbon dioxide insufflation, a technique for adrenal imaging available at that time [2], revealed no enlargement of the patient's adrenal. Subsequently the patient withdrew from further medical evaluation and management, her whereabouts remained unknown until 25 years later, when she presented with intracranial haemorrhage and ESKD [3]. Genetic analysis demonstrated a compound heterozygosity with two mutations in the gene encoding the enzyme 11 β -hydroxysteroid dehydrogenase type 2 (11 β HSD2) [3], an enzyme which plays a crucial role in

mineralocorticoid regulated renal sodium transport. The underlying defect affecting this girl was a condition that was characterized in the late 1970s by Ulick and New and that was dubbed apparent mineralocorticoid excess (AME) syndrome [4,5], because it mimicked mineralocorticoid excess, but was not caused by a steroid with mineralocorticoid action such as aldosterone or deoxycorticosterone. It is now clear that AME is caused by mutations in the gene encoding the enzyme 11 β HSD2. In this overview we discuss the subsequent cloning, physiology, pathophysiology, and clinical relevance of the 11 β -hydroxysteroid dehydrogenase (11 β HSD) enzymes.

2. Unmasking a new biological principle

The biochemical characterisation of the severe juvenile low-renin hypertension termed apparent mineralocorticoid excess (AME) by New et al. [4,5] unveiled a new biological principle. The classical view of hormone action in target organs is that of hormone signalling by the binding of ligand to a specific, cognate receptor. However, in mineralocorticoid target organs, hormonal specificity is determined by an enzyme and not the receptor.

When aldosterone enters the cell, it binds to the mineralocorticoid receptor (MR), and thereafter the ligand-receptor complex is translocated into the nucleus. Binding to hormone response elements

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(HRE) increases the transcription of genes encoding specific aldosterone-inducible proteins, such as rate-limiting subunits of the apical epithelial sodium channel (ENaC) (Fig. 1A). In response sodium influx causes intracellular sodium (Na^+) to rise; this increase in substrate increases turnover of Na^+/K^+ ATPase, which in turn increases Na^+ reabsorption and potassium (K^+) excretion. Activation and induction of these Na^+ transporters is crucial to unfold the physiological role of the aldosterone system, which is to counteract excessive water and salt loss and maintain normotension in the face fluid losses. On the other hand, unrestrained activation of the MR, as occurs in many forms of mineralocorticoid hypertension, causes excessive Na^+ reabsorption leading to volume expansion and contributes to hypertension.

In vitro binding studies demonstrate that cortisol and aldosterone have the same affinity for MR, although in mineralocorticoid target organs only aldosterone acts as the physiologic agonist (Fig. 1B) [6]. The MR is protected from activation by glucocorticoids through the agency of a gatekeeper enzyme, the microsomal 11β -hydroxysteroid dehydrogenase type 2 enzyme ($11\beta\text{HSD2}$), evidence that mineralocorticoid target tissue specificity is enzyme, not receptor, mediated (Figs. 1 and 2) [7]. MR and glucocorticoid receptors (GR) have a high degree of sequence identity [8], 94% in the central DNA-binding domain and 57% in the C-terminal ligand-binding domain. With the cloning and expression of the MR [8] it was confirmed that aldosterone and cortisol have similar binding affinities for MR *in vitro* [9,10]. Circulating levels of cortisol in humans and corticosterone in rodents

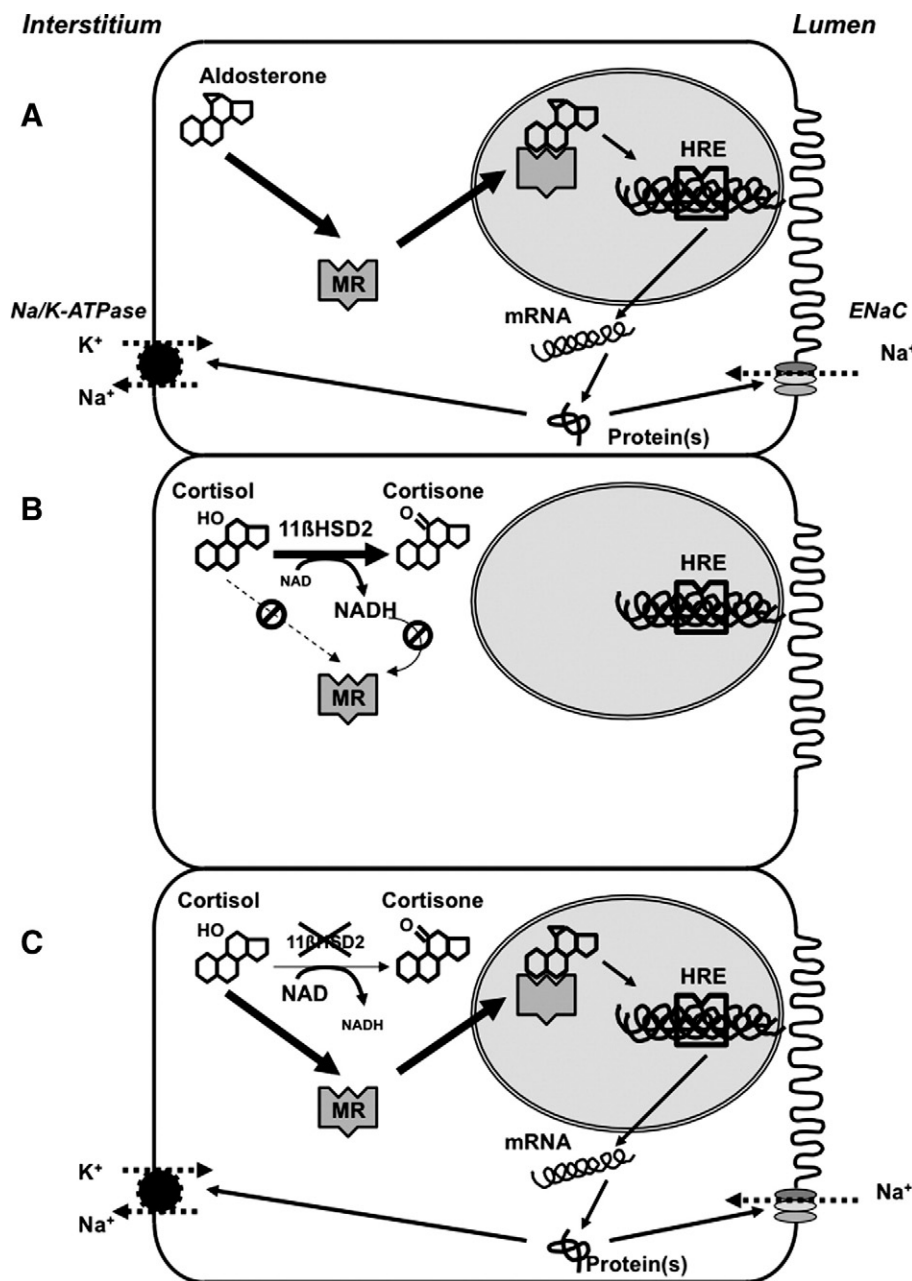


Fig. 1. Mineralocorticoid action in renal cells of the cortical collecting duct (CCD): (A) When aldosterone enters the CCD cell, it binds to the mineralocorticoid receptor (MR), and thereafter the ligand-receptor complex is translocated into the nucleus. Binding to its hormone response element (HRE) increases the transcription of genes encoding specific aldosterone-inducible proteins, such as the rate-limiting subunits of the apical epithelial sodium channel (ENaC), sgk-1 and basolateral Na^+/K^+ ATPase. In turn, this stimulates sodium (Na^+) reabsorption and potassium (K^+) excretion. (B) When cortisol enters the CCD cell the enzyme 11β -hydroxysteroid dehydrogenase type 2 ($11\beta\text{HSD2}$) largely (~90%) converts it to inactive cortisone and generates high levels of NADH, thereby protecting the MR by from activation by cortisol. C. When $11\beta\text{HSD2}$ activity is decreased as a consequence of inherited or acquired reduction in enzymatic activity, the intracellular cortisol level increases further, but the level of NADH plummets, leading to activation of the MR by cortisol. The resulting glucocorticoid activation of MR produces Na^+ retention, volume expansion and hypertension.

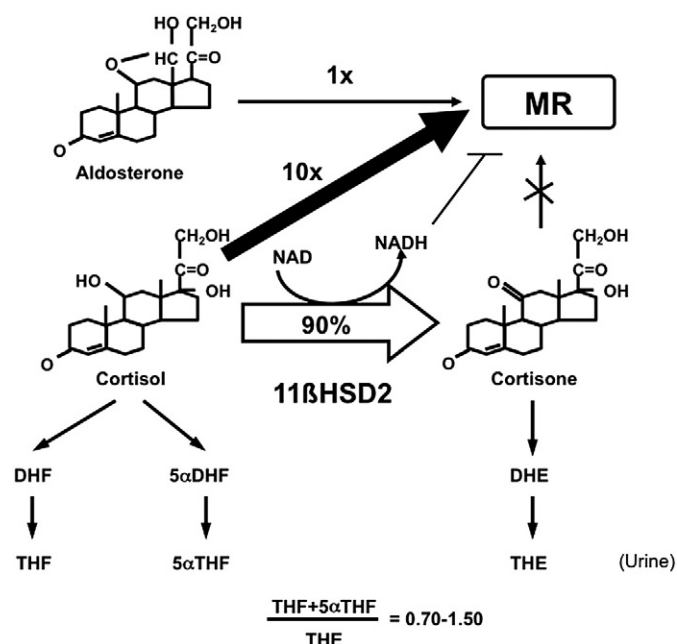


Fig. 2. Peripheral cortisol metabolism and mineralocorticoid receptor selectivity: cortisol and aldosterone bind with equal affinity to the mineralocorticoid receptor (MR). Plasma concentrations of cortisol are 1000× higher than those of aldosterone, but in MR-target cells the NAD-dependent 11β-hydroxysteroid dehydrogenase type 2 (11βHSD2) debulks intracellular cortisol and generates high levels of NADH. Generated NADH maintains cortisol–MR complexes in an inactive state and permits aldosterone to selectively activate the receptor, despite 10× higher intracellular cortisol levels. The hemiacetal conformation of the 11-hydroxyl group with the 18-aldehyde group of aldosterone renders this steroid a poor substrate for the enzyme. Cortisol and cortisone are substrates for a series of enzymatic activities in the liver. These include reduction of Δ^4 double bond yielding 5α- and 5β-dihydrocortisol (DHF) and dihydrocortisone (DHE), and reduction of 3-keto group, producing 5α- and 5β-tetrahydrocortisol (THF) and tetrahydrocortisone (THE). The activity of the 11βHSD2 enzyme can be assessed *in vivo* by measuring the urinary (THF + 5αTHF)/THE ratio by gas chromatography and mass spectrometry. In normal subjects this ratio ranges between 0.7 and 1.5.

are three orders of magnitude higher than aldosterone levels, despite which *in vivo* only aldosterone acts as a physiologic agonist of the MR. This paradox was apparently solved by the discovery of the enzyme 11βHSD [7,10], which converts biologically active 11-hydroxysteroids to their inactive 11-keto-steroid forms, thus conferring ligand specificity on MR; in addition, it stoichiometrically generates NADH, which appears to be crucial in maintaining glucocorticoid-occupied MR in an inactive state, by mechanisms which remain to be established [11]. Cyclisation of the 11-hydroxyl group with the 18-aldehyde group of aldosterone renders the physiological mineralocorticoid resistant to 11βHSD. Of the two cloned 11βHSD isoforms, only the type 2 (11βHSD2) acts in this way to protect MR. When 11βHSD2 activity is decreased, reflecting inhibition or mutation, cortisol is able to stimulate MR and induces the state of apparent mineralocorticoid excess (Fig. 1C).

3. 11βHSD cloning, tissue localization and biochemistry

The 11βHSD enzymes catalyse the interconversion of cortisol and cortisone in humans (Fig. 2), and of corticosterone and dehydrocorticosterone in rodents [7]. Cortisone and dehydrocorticosterone show minimal biological activity per se, reflecting their negligible affinity for GR and MR. Two kinetically distinct forms of 11βHSD (11βHSD1 and 11βHSD2) have been cloned and are differentiated by directionality *in vivo*, cofactor specificity and tissue expression (Table 1) [12–16]. 11βHSD1 activity and expression is found in most tissues; its K_m for cortisol is more than an order of magnitude higher than that of 11βHSD2; it is reduced nicotinamide adenine dinucleotide phosphate (NADPH)-preferring, and has been shown to have overwhelmingly

reductase activity *in vivo* (Table 1). Mice lacking 11βHSD1 have elevated circulating levels of plasma corticosterone and show reduced glucocorticoid responses including blunted gluconeogenesis in response to fasting, and improved lipid profiles, but no hypertension. [17–19].

In contrast, 11βHSD2 has been identified in a limited range of tissues primarily involved in transepithelial sodium transport [14–16]. It has a low K_m for cortisol (40–50 nM), and even lower for corticosterone (~4 nM), is nicotinamide adenine dinucleotide (NAD)-requiring and shows only dehydrogenase activity for endogenous glucocorticoids (Fig. 2, Table 1) [14–16], although reduction of dehydrodexamethasone has been demonstrated *in vitro* [20,21].

Immunohistochemical studies have consistently localized 11βHSD2 to a restricted range of organs, primarily expressing sodium-transporting epithelia such as distal renal tubules, distal colon, sweat and salivary glands, but also placenta and vascular wall [14–16,22,23]. Odermatt et al. [24] co-expressed epitope-tagged MR and 11βHSD2 in HEK-293 cells lacking 11βHSD2 activity and analysed their subcellular localization by fluorescence microscopy. When co-expressed with 11βHSD2 the MR displayed a reticular distribution pattern, absent ligand, suggesting association of MR with 11βHSD2 at the endoplasmic reticulum membrane. Aldosterone induced rapid nuclear translocation of MR, with the enzyme remaining tethered in the cytoplasmic compartment. In parallel studies [25], green fluorescent protein (GFP) tagged MR were expressed in similar cells, and absent steroid MR were similarly shown to be predominantly cytoplasmic. Addition of aldosterone, corticosterone or spironolactone all served to move MR intranuclear—rapidly with aldosterone, less rapidly with corticosterone, slowly and only partially with spironolactone [25].

11βHSD2 in the kidney is primarily expressed in the cells of the cortical collecting duct [23,26,27]; in contrast, the liver is the principal site for cortisone to cortisol conversion by 11β-hydroxysteroid dehydrogenase type 1 (11βHSD1). Both cortisol and cortisone are substrates for a series of enzymatic activities in the liver, including the reduction of Δ^4 double bond to reduction of 3-keto group and reduction of 20-keto group, most of which metabolites are excreted in the urine as glucuronides, with only a small part excreted unconjugated, mainly as 3-oxo-4-ene steroids [28]. The activity of the 11βHSD2 enzyme can be reliably assessed *in vivo* by measuring the ratio of biologically active cortisol (F) to inactive cortisone (E), or their tetrahydrometabolites (THF and THE), in the urine by gas chromatography with mass spectrometry [29,30]. Increase in urinary free F/E

Table 1
11β-hydroxysteroid dehydrogenase (11βHSD) isoenzymes.

	11βHSD type 1	11βHSD type 2
<i>Molecular biology</i>		
Chromosome	1	16
Gene structure	30 kb, 6 exons	6.2 kb, 5 exons
mRNA	1902 bases	1944 bases
Amino acids	292, 34 kDa	405, 45 kDa
<i>Enzyme kinetics</i>		
Activity	bi-directional, reductase <i>in vivo</i>	dehydrogenase only
Substrate affinity	low (K_m μM range)	high (K_m nM range)
Cofactor specificity	NADP/NADPH	NAD
<i>Expression</i>		
Tissue localization	ubiquitous, mainly liver, lung	kidney, colon, sweat glands
	adipose tissue, brain, gonads	placenta
Cellular localization	microsomal	microsomal, MR positive cells
Function	elevation of cellular cortisol concentration	protection of the MR

MR = mineralocorticoid receptor.

or urinary (THF + 5 α THF)/THE ratio thus indicate decreased 11 β HSD2 activity [31].

4. Adrenal steroids, renal salt handling and blood pressure regulation

4.1. Aldosterone, blood pressure and salt

Normal regulation of fluid and electrolyte homeostasis in mammals is regulated in a negative-feedback loop by the renin–angiotensin–aldosterone system [32], and by the direct effects of plasma [K⁺] on aldosterone secretion [33]. Renin is released by the juxtaglomerular cells of the afferent arterioles and macula densa cells of the kidney, and aldosterone is produced by the adrenal glands. Plasma concentrations of renin and angiotensin II rise in response to contraction of intravascular volume and reduction in renal perfusion, and are lowered by intravascular volume expansion. To maintain blood pressure and organ perfusion healthy subjects respond to volume loss by an increase in renin and aldosterone secretion, which minimises urinary Na⁺ losses and enhances water retention, and respond to K⁺ loading by a direct effect on the adrenal to raise aldosterone secretion rate and thus K⁺ excretion. Complex organisms on the planet evolved in the Na⁺-rich aqueous environment of the sea [34], and terrestrial life thus necessitated the development of mechanisms to retain Na⁺ [35]. Fractional regulation of salt and water balance involves the kidney [36,37], and in particular the renal medulla, which develops very differently between species, and is more prominent in species with a high urinary concentrating capacity [38]. Nephron elements such as the loop of Henle and the collecting duct, which function to concentrate the urine by increasing Na⁺ and water reabsorption, are found in humans and other terrestrial mammals, but are absent or rudimentarily developed in fish and amphibians. This evolutionary adaptation of the nephron underlines the central role of the kidney in the homeostatic regulation of Na⁺ and water balance [35].

Most species, including humans, evolved in a Na⁺ poor, K⁺ rich context [36,37], and thus needed to retain as much dietary salt as possible, and conversely to shed potassium. It has been estimated that in the first 10 million years of existence, as hunters and gatherers, humans enjoyed a dietary salt intake of approximately 1 g daily (~17 meq). Given the variable availability of salt, particularly inland, humans developed regulatory mechanisms to prevent loss of Na⁺ through the kidney and other epithelia, in which aldosterone, MR and 11 β HSD2 are crucial elements. It is important to note that MR preceded terrestrial evolution and are clearly present in fish, which lack the capacity to make aldosterone. Cortisol is thus the evolutionary driver for MR [39]; a possibly useful question might thus be why epithelial Na⁺/K⁺ balance regulation pressed into service an existing receptor, which recognised a ‘novel’ ligand (aldosterone) with affinity equivalent to that for cortisol, protected in epithelia by the co-expression of 11 β HSD2 at very high levels, rather than evolving an aldosterone-specific MR. Species that are able to transport or mine salt, or balance Na⁺ and K⁺ intake by meat eating, appear unable to suppress aldosterone to a degree sufficient to obviate a pattern of deleterious effects seen experimentally magnified in animals administered exogenous aldosterone and maintained on 0.9% NaCl solution to drink [40]. This possible inability to lower aldosterone appropriately in the face of a normal (Western) Na⁺ intake (150–500 meq/day), coupled with an unrestricted Na⁺ appetite, may thus be a major contributing factor to human cardiovascular disease [41].

4.2. Cortisol and blood pressure

Glucocorticoid (GC) treatment and endogenous cortisol excess as seen in Cushing's syndrome have a profound effect on blood pressure [42]. The exact mechanisms mediating cortisol-induced hypertension

are still not fully understood. The pathogenesis of hypertension in Cushing's syndrome is multifactorial and may involve the following factors: (a) the activation of the MR by cortisol excess in the distal kidney tubule, leading to excess sodium Na⁺ retention; (b) an enhanced hepatic production of angiotensinogen, providing increased abundance of renin substrate [43,44]; and (c) an increased peripheral vascular sensitivity to adrenergic agonists [45]. It is obvious that excess renal sodium Na⁺ retention and the ensuing increase in blood volume characterize the primary mechanism for GC-associated hypertension. This is the consequence of excess MR stimulation by supra-physiologic cortisol concentration and the failure of the enzyme 11 β HSD2 fails to adequately convert cortisol into the inactive form cortisone because substrate availability is greater than the rate of conversion to its product. Kinetic properties of 11 β HSD2 suggest that saturability of this enzyme can already be achieved at high-normal physiologic plasma cortisol levels [20,46]. As a result, stimulation of the MR by cortisol leads to renal Na⁺ retention, volume expansion and finally to an increase in blood pressure with suppression of renin secretion. In patients with pituitary and particularly with ectopic Cushing's syndrome, defective 11 β HSD2 activity can be demonstrated by an increased urinary cortisol to cortisone ratio [29,47]. Correction of hypertension by kidney transplantation in a patient with AME [48] argues in favour of a central role of the kidney in the pathogenesis of GC-associated hypertension related to 11 β HSD2 deficiency.

However, some observations suggest that mechanisms independent from Na⁺ retention play an important role in glucocorticoid-associated hypertension. It has been shown that the Na⁺ retention and increase in blood pressure induced by exogenous cortisol may not be fully reversed by spironolactone [49,50]. Since spironolactone acts by competitive inhibition of binding to MR [51], it is possible that the doses of cortisol given in these studies [49,50] were sufficiently high to produce circulating levels that compete with spironolactone for MR. Nevertheless, other renal or extrarenal effects of cortisol upon GR have to be considered. There is good evidence that exogenous cortisol potentiates vasopressor responses to angiotensin II and catecholamines [52] and recent studies have shown that corticosterone enhances phenylephrine-induced contraction of intact aortic rings via induction of mitogen-activated protein kinases [53]. There is also evidence implicating the MR in the central nervous system in the pathogenesis of mineralocorticoid hypertension [54,55]. In animal experiments, intracerebroventricular administration of very low doses of aldosterone can induce hypertension and this effect can be progressively blocked by 1- to 2-fold ICV corticosterone [56], thus suggesting that there may be no pathophysiological role for 11 β HSD. Thus, future investigations in this field will need to take into account a wider spectrum of mechanisms including vasoconstriction and angiotensin II response.

5. 11 β HSD2 and mineralocorticoid hypertension

A markedly reduced activity of 11 β HSD2 can be caused by mutations in the *HSD11B2* gene or inhibition of this enzyme by xenobiotics such as licorice. The clinical syndromes ensuing from *HSD11B2* gene mutations or inhibition of 11 β HSD2 by exogenous compounds are indistinguishable and present as mineralocorticoid hypertension with hypokalaemia, metabolic alkalosis and suppressed circulating renin and aldosterone. Hypertension is primarily caused by sodium and fluid retention secondary to excess activation of the MR by cortisol.

5.1. Apparent mineralocorticoid excess (AME)

Inherited deficiency in 11 β HSD2 causing impaired peripheral metabolism of cortisol and presenting with juvenile hypokalaemic hypertension is known as the syndrome of apparent mineralocorticoid excess (AME) [1,4,5]. Typical clinical signs and symptoms of AME

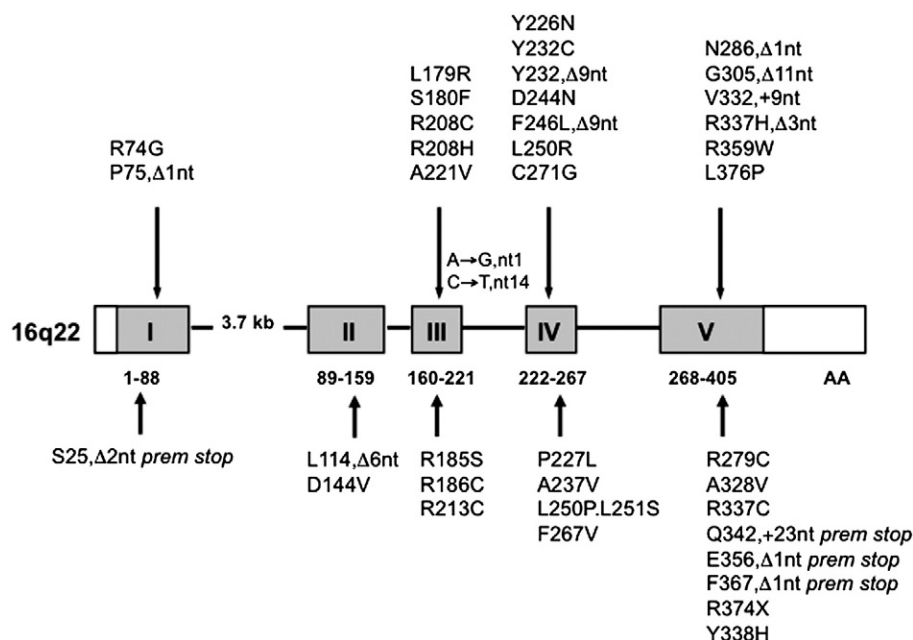


Fig. 3. Location of HSD11B2 gene mutations: The HSD11B2 gene is located on chromosome 16 and consists of 5 exons, labelled I through V (grey boxes). Introns are represented by the lines and the untranslated regions by the open boxes. Numbers below the exons indicate the amino acid (AA) number. Mutations are listed relative to their position in the gene. Those shown below the gene are mutations investigated by the author, with mutations reported above the gene reported by others.

include low birth weight and failure to thrive, severe hypertension, hypokalaemia, suppressed plasma renin activity with hypoaldosteronaemia, polyuria, polydipsia and nephrocalcinosis. The disease is inherited as an autosomal recessive trait, and various mutations in the HSD11B2 gene have subsequently been shown to cause 11 β HSD2 deficiency. Signs and symptoms of the syndrome can be partially or fully reversed by treatment with the MR-antagonist spironolactone [4,57–59] or kidney transplantation [48], evidence for the crucial role of the renal 11 β HSD2 in MR protection [26,27]. Normally plasma cortisol levels are in the sub-micromolar range, while aldosterone levels are sub-nanomolar; even in 11 β HSD protected cells intracellular levels of glucocorticoid are $\sim 10\times$ those of aldosterone [60]. In AME, deficient 11 β HSD2 enzyme activity further elevates intracellular glucocorticoid levels, but crucially does not generate the levels of NADH needed to keep the cortisol–MR complexes inactive. The resultant MR activation produces Na^+ retention, volume expansion, hypokalaemia and suppression of plasma renin and aldosterone secretion. Thus, the hallmark of the disease is a state of excess mineralocorticoid activity in the absence of aldosterone, and the abnormal urinary steroid profile of an increased ratio of cortisol to cortisone metabolites, or urinary free cortisol to cortisone.

Mutations in the HSD11B2 gene have been unequivocally shown to be the molecular basis of the syndrome of AME [46,61–77] (Fig. 3). Most of the known mutations are found in exons 3, 4 or 5 of the HSD11B2 gene, with the exception of the R74G and P75,Δ1nt in exon 1 [76] and L114,Δ6nt mutant in exon 2 [73]. A few mutations were found to leave the amino acid sequence unchanged, but potentially to cause aberrant splicing; sequence analysis of a *de novo* base transversion, 771C>G in exon 4 (V254V, TGC to TGG) creates a canonical donor splice site [76].

Although patients with homozygous mutations from different families show varying degrees of severity in terms of clinical and biochemical features (Fig. 4; personal observation and refs [46,62,64,67,69,70,73]), most patients with classic AME syndrome have characteristic signs of severe 11 β HSD2 deficiency (Fig. 4). In those instances, birth weights are significantly lower than that of their unaffected sibs, and the patients are short, underweight and hypertensive for their age. The *in vivo* activity of 11 β HSD2, as assessed by urinary excretion ratio of the cortisol (THF + 5 α THF) to

cortisone (THE) metabolites, is characteristically abnormal, with ratios of 6–60, whereas the normal ratio is ~ 1.0 . *In vitro* expression studies showed that most of the mutations described result in an 11 β HSD2 protein with absent enzymatic activity (Fig. 4) [46,61–64,66,68,71,76]. The index case reported by Werder 35 years ago [1] was found to have a mutation at Tyr(338) of HSD11B2 [3]. This Tyr(338)His mutation

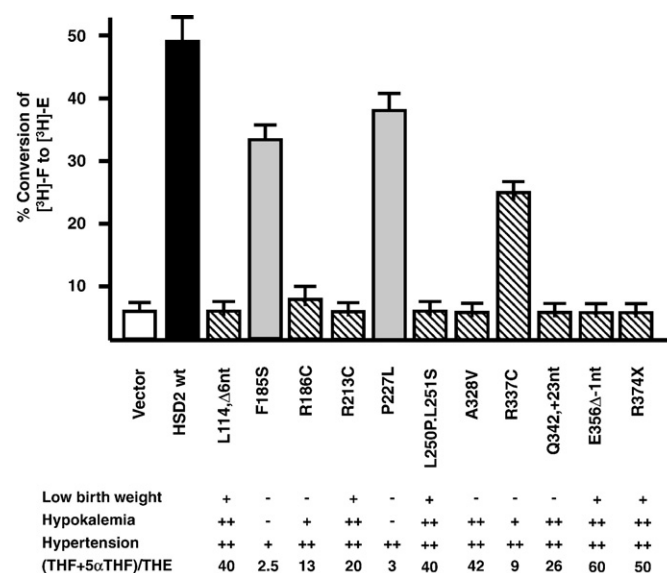


Fig. 4. Genotype–phenotype correlations in apparent mineralocorticoid excess (AME): The bars show the results of *in vitro* enzymatic activity assays using CHOP cells transfected with mutant plasmids of the 11 β -hydroxysteroid dehydrogenase type 2 (HSD11B2) gene. Transfected cells were incubated for 2 hours with [3 H]-cortisol, with [3 H]-cortisol and [3 H]-cortisone from the supernatant at the end of incubation separated by chromatography and quantified. Birth weight, blood pressure, serum potassium and the urinary (THF + 5 α THF)/THE ratio as a measure of *in vivo* 11 β HSD2 activity are correlated with the genotype and *in vitro* 11 β HSD2 activity. When *in vitro* enzymatic activity is completely abolished as a consequence of mutations in the HSD11B2 gene carriers of the mutation almost invariably show classical AME with severe hypokalaemia, hypertension and a markedly increased (THF + 5 α THF)/THE ratio. In milder cases with isolated hypertension the identified mutation produces only a moderate reduction in 11 β HSD2 activity.

results in an 11 β HSD2 enzymatic activity that is partially retained at 26 °C or in the presence of the chemical chaperones glycerol 5% and dexamethasone at 1 μ M concentration, indicating thermodynamic instability and misfolding. Experiments with the proteasomal inhibitors MG132 and lactacystin provided supplementary evidence that the degradation of 11 β HSD2 occurs through the proteasome pathway [3]. Therefore, in some individuals with AME impaired 11 β HSD2 protein stability rather than reduced gene expression or loss of catalytic activity seems to be responsible for the development of hypertension.

It has become evident that depending on the degree of loss of enzyme activity, 11 β HSD2 mutations can cause a spectrum of hypertension ranging from a severe, life-threatening disease in early childhood to a milder form diagnosed only in adults. Mantero et al. described a milder form of AME, which they labelled AME type II [72,78]. In AME type II the urinary ratio of (THF + 5 α THF)/THE is almost normal, with the main abnormality in cortisol metabolism defective in A-ring reduction presenting as prolonged plasma half-life of [11 α -³H]-cortisol [78]. As in classic AME; molecular analysis of a large pedigree with AME type II from Sardinia showed a C945T mutation, resulting in the substitution of a cysteine for an arginine at codon 279 (R279C mutation) in 11 β HSD2. *In vitro* expression of the mutant enzyme showed an identical K_m for cortisol as the wild-type enzyme, while the maximum velocity was reduced by approximately 35% [79]. An even more subtle form of AME was reported by Wilson et al. [70] a decade ago, where a case of a young teenager with low-renin hypertension, but without the phenotypic features of AME was found to be caused by a mutation in HSD11B2 producing mild deficiency in 11 β HSD2 activity. From clinical observations and *in vitro* co-transfection studies 11 β HSD2 activity appears mildly impaired in heterozygotes [64], predisposing to isolated hypertension of later onset. Heterozygous parents of children with AME have been reported to suffer from hypertension with no other characteristic signs of AME except from slightly raised urinary (THF + 5 α THF)/THE ratios [64,68].

5.2. Licorice-induced hypertension

Licorice root has been used in Europe since prehistoric times, and is well documented in written form from the time of the ancient Greeks. Licorice root and extracts have been used in medical herb products, sweeteners and mouth fresheners [80]. The active ingredient of licorice is glycyrrhizic acid, which is hydrolysed into its aglycone glycyrrhetinic acid *in vivo*. Licorice products are made from peeled and unpeeled dried root, powdered or finely cut; these formulations have different concentrations of glycyrrhizic acid, which can vary from trace amounts to 20% based on the extraction process. Carbenoxolone, the hemisuccinate of glycyrrhetinic acid, was previously successfully used to treat patients with peptic ulceration [81]. Licorice possesses additional endocrine effects including glucocorticoid activity, antiandrogen effects, and estrogenic activity [81–83]. Patients consuming excessive quantities of licorice present with hypertension and hypokalaemia [83–85], which may be severe enough to cause myopathy and cardiac arrhythmias. Both renin and aldosterone levels are suppressed [31] and exchangeable Na⁺ levels are increased. The condition responds to spironolactone and is reversible upon stopping licorice ingestion [31,86]. Glycyrrhizic and glycyrrhetinic acids have very low affinity for MR, but are very potent competitive inhibitors of 11 β HSD2 (K_i of approximately 5–10 nM) [87]. Licorice administration to normal volunteers results in a mineralocorticoid excess state, an increase in the urinary (THF + 5 α THF)/THE ratio, an increase in plasma cortisol half-life, and a decrease in circulating cortisone values, indicative of inhibition of 11 β HSD2 *in vivo* [31,88]. It is now clear that licorice induces an acquired and milder form of AME, causing its mineralocorticoid effects through inhibition of 11 β HSD2 rather than directly occupying and activating MR.

6. Role of 11 β HSD2 in “essential” hypertension

The relationship between blood pressure, mineralocorticoid effect and salt sensitivity is complex and any attempts to assess a possible role of impaired 11HSD2 activity as a contributing element to hypertension or a salt-dependent driver of blood pressure increments have to be interpreted with caution. Excess circulating aldosterone *per se* does not cause hypertension in settings of sodium depletion, as is the case in Gitelman's syndrome [89]. This condition is caused by loss of function mutations in the Na-Cl cotransporter of the distal convoluted tubule [90]. It features inherited salt-loss, hypokalaemic alkalosis and excess circulating renin and aldosterone level, but normal blood pressure [89,91]. These findings demonstrate that the relationship between mineralocorticoid activity and blood pressure has to be assessed in the context of total body sodium balance.

Hypertension with hypokalaemia, metabolic alkalosis and suppressed plasma renin activity defines mineralocorticoid hypertension. Mineralocorticoid hypertension is the consequence of an overactivity of the epithelial sodium channel of renal cells in the distal nephron and usually occurs when the MR is activated by aldosterone. Clearly, most patients with essential hypertension lack overt signs of mineralocorticoid excess and do not have suppressed plasma renin or low serum potassium. However, some hypertensive patients demonstrate subtle biochemical changes suggestive of a mineralocorticoid effect contributing to hypertension such as low renin levels, a positive correlation between blood pressure and serum Na⁺ levels, or a negative correlation with potassium [92]. Because subjects with severely impaired 11 β HSD2 activity present with overt features of mineralocorticoid hypertension, it is tempting to hypothesise that more subtle changes in 11 β HSD2 activity could contribute to hypertension in a subset of essential hypertensive patients with low renin hypertension.

7. 11 β HSD2 activity in “essential” hypertension

As described previously, *in vivo* 11 β HSD2 activity can be assessed by measuring the ratio of active to inactive glucocorticoids in the urine; an increase in urinary free F/E or (THF + 5 α THF)/THE ratio indicates decreased 11 β HSD2 activity [31], similarly a prolonged half-life of plasma cortisol demonstrates reduced oxidation of cortisol to cortisone. Some studies have reported that the half-life of cortisol is significantly prolonged, the excretion of urinary cortisol metabolites increased and the vasoconstrictor response to glucocorticoids enhanced in some patients with essential hypertension [93,94]. In the “4 corner study” impaired conversion of cortisol to inactive metabolites has also been reported in young men with higher blood pressure whose parents also had high blood pressure [95], and another study found a positive association between urinary free cortisol and salt-resistant hypertension [79]. It has also been demonstrated that age significantly and positively correlates with the ratios of (THF + 5 α THF)/THE and F/E, suggesting that reduced 11 β HSD2 activity may be a previously unrecognized risk factor contributing to the rising prevalence of arterial hypertension in elderly [96]. This observation is corroborated by the report of 11 β HSD2 impairment in Japanese elderly patients with mineralocorticoid excess state [97]. Moreover, an impaired 11 β HSD2 activity can predispose to hypertension induced by exogenous glucocorticoids, as shown by Olivieri et al. [98]. These authors showed that a urinary (THF + 5 α THF)/THE ratio >1.5 was associated with a 3.8-fold incremental risk of hypertension after prednisolone therapy, regardless of duration and intensity of exogenous corticosteroid therapy [98]. Thus, taken together, these studies support the view that an impaired 11 β HSD2 activity may play some role in a subset of patients with essential hypertension. Nevertheless, other authors could not confirm these observations [99,100]. There are several possible explanations for this discrepancy, notably the differences in the ethnic groups, age, selection criteria and

the methods used to assess 11 β HSD2 function. The observation, in some but not all studies, of an apparent reduction in 11 β HSD2 activity in hypertensive subjects gives rise to many questions. Firstly, is this correlative association of pathogenetic value or not? Secondly, if indeed mildly impaired 11 β HSD2 activity plays a pathogenetic role, what degree of 11 β HSD2 impairment is necessary to induce hypertension? Thirdly, what causes impaired 11 β HSD2 activity in these subjects? Unfortunately, there are no unequivocal answers to these questions. Furthermore, because salt plays a permissive role, it is difficult to establish whether there is a linear relationship between 11 β HSD2 activity and blood pressure.

Since steroid hormones modulate renal Na⁺ retention, it is possible that variations in 11 β HSD2 activity may be responsible for the sensitivity of blood pressure to changes in dietary salt intake. A salt-sensitive response of blood pressure has been observed in not only patients with hypertension, but also in some young normotensive individuals [101]. These subjects also display a number of traits, including suppression of the renin-angiotensin system, that can also be found in some patients with essential hypertension [102]; it has therefore been suggested that salt-sensitive individuals may be genetically predisposed to the development of hypertension [103]. Our own data suggest that impaired 11 β HSD2 activity is associated with an increased susceptibility of blood pressure to salt load [104], an observation in line with the well-established concept that low-renin hypertension is generally considered a salt-sensitive form of high blood pressure [103]. These findings are in apparent contrast with the observation of Litchfield et al. [79], that subjects with highest urinary free cortisol show the least sensitivity of blood pressure to dietary salt. In this study urinary free cortisol (but not cortisone or (THF + 5 α THF)/THE ratios) were measured, thereby not allowing a direct evaluation of 11 β HSD2 activity; reduced 11 β HSD2 activity correlates with a decrease urinary excretion of free cortisone rather than an increased urinary free cortisol excretion [29]. Thus, measuring cortisone and its metabolites or (THF + 5 α THF)/THE in the urine seems to be the most appropriate assay of renal 11 β HSD2 activity. Unfortunately, adequate measurements of urinary cortisol and cortisone metabolites are not readily available and thus unsuitable for routine screening. Other observations on the metabolism of GC hormones seem to link 11 β HSD2 function to hypertension. Low-birth weight and/or stillbirth [67] are often found in patients with AME. It seems likely that 11 β HSD2 protects the foetus against excessive levels of maternal glucocorticoids [105,106]; deficient foetal (placental) 11 β HSD2 would allow increased levels of glucocorticoids to cross the placenta and thus inhibit foetal growth. In humans, low birth weight is a risk factor for the development of essential hypertension in adult life [107–109]; some clinical and experimental observations suggest that decreased activity of placental 11 β HSD2 reduces birth weight and produces hypertensive adult offspring [49,108,109]. A mild form of 11 β HSD2 deficiency could therefore present as a low birth weight infant with the onset of hypertension in later life.

7.1. 11 β HSD2 gene in “essential” hypertension

It is plausible that mutations leading to decreased 11 β HSD2 activity can masquerade as essential hypertension and therefore that a subset of patients among the essential hypertensive population may suffer from a subtle form of AME. Non-conservative mutations in the *HSD11B2* gene may not necessarily result in severely impaired 11 β HSD2 activity presenting with overt AME [70]. Wilson et al reported on a girl with a homozygous *HSD11B2* gene mutation resulting in hypertension and suppressed plasma renin levels, but no significant hypokalaemia or the other phenotypic features that could support the diagnosis of AME [70]. Li et al. [68] reported that heterozygous parents of a child with AME have hypertension and increased urinary (THF + 5 α THF)/THE ratio, but no other overt features of AME. Generally though, the phenotypic effects of the

majority of mutations in the *HSD11B2* coding region in AME are quite striking. Thus, if an inherited abnormality in 11 β HSD2 is to play a role in some patients with hypertension it is more likely that this may be due to reduced expression of the gene, rather than expression of an enzyme with altered amino acid sequence.

There have been few attempts to analyse whether an association exists between *HSD11B2* gene mutations, 11 β HSD2 activity and essential hypertension. The first attempt by Watson et al. [110] was rather circumstantial. These authors reported a genetic association of a *HSD11B2* flanking microsatellite and hypertension in African-Americans with ESKD [110]. They argued that because one of the leading causes of ESKD in African-Americans is low-renin hypertension it is conceivable that impaired 11 β HSD2 activity might be more prevalent among this population. An association between the same Glu¹⁷⁸/Glu (G534A) polymorphic marker in exon 3 of *HSD11B2* and ESKD in Caucasians was also described, although this marker is not associated with essential hypertension in humans [111]. Since this mutation does not alter the amino acid sequence, and thus the activity of 11 β HSD2, the mechanisms underlying the association of ESKD with this polymorphic marker are unclear.

Structural analysis of the *HSD11B2* gene in patients with ESKD demonstrated that the frequency of homozygosity for mutated alleles of the gene is <1/250,000 in Caucasians [112]. Others have also failed to demonstrate the presence of mutations in the *HSD11B2* coding region and hypertension in an unselected hypertensive population. Kamide et al. [113] sequenced the entire coding region and the promoter region of *HSD11B2* in 953 Japanese hypertensive patients and identified a series of genetic variations in *HSD11B2*. They found 5 missense mutations in 11 patients and 1 novel frameshift mutation in a heterozygous state, in addition to 19 genetic variations. All genetic variations identified were rare, with minor allele frequencies less than 0.005. Four missense mutations, L14F, R74H, R147H, and R335H, were identified in hypertensive and normotensive subjects with a similar frequency, suggesting that these missense mutations may not strongly affect the etiology of essential hypertension [113]. These results indicate that missense mutations in *HSD11B2* do not substantially contribute to essential hypertension in Japanese.

Brand et al. [114] analysed a polymorphic CA-repeat microsatellite marker near the *HSD11B2* gene in a large series of families with essential hypertension, but found no correlation between this marker and blood pressure in their cohort. We analysed the same polymorphic marker in salt-sensitive subjects and found a positive association between the short allele A7 homozygosity and salt-sensitivity, and a negative correlation with allele pair A7/A8 of the microsatellite marker [104], findings later confirmed by Agarwal et al. [115]. This suggests that the activity of the 11 β HSD2 enzyme may be genetically determined by variants in the *HSD11B2* promoter or by the presence of undetected mutations in the *HSD11B2* gene itself, an issue deserving further investigation. These genetic findings on *HSD11B2* polymorphisms suggesting that abnormal regulation of the gene may promote hypertension are backed by the observation that salt-sensitive subjects show higher cortisol levels after mental stress [116]. The apparently divergent findings of some of these studies may be explained by the lack of selection for the blood pressure response to salt-load in the patients studied by Brand et al. [114].

Two different polymorphisms of the *HSD11B2* gene, Thr156/Thr (C468A) in exon 2 (ex2) and Glu178/Glu (G534A) in exon 3 (ex3), have been shown to be significantly more prevalent in hypertensive subjects than in control subjects [117]. The role of these polymorphisms has not been further investigated, but it has been proposed that they may be related to mutations in the promoter region of *HSD11B2* or to the modulation of 11 β HSD2 mRNA processing in hypertensive subjects. This view could be supported by the observation by Agalou et al. [118] that circulating mRNA levels for 11 β HSD2 are significantly lower in hypertensive patients than in healthy subjects, suggesting that measurement of mRNA for 11 β HSD2 in hypertension may

identify salt-sensitive subjects. The role of human 11 β HSD2 in relation to hypertension is more likely to occur at the level of HSD11B2 epigenetic control. Friso et al. [119] found that elevated HSD11B2 promoter methylation is associated with hypertension developing in glucocorticoid-treated patients in parallel with a higher urinary (THF + 5 α THF)/THE ratio. They also showed an association between elevated urinary (THF + 5 α THF)/THE ratio and HSD11B2 promoter methylation in essential hypertensive patients, suggesting that there is a link between the epigenetic regulation through repression of HSD11B2 and hypertension [119]. Future prospective studies comparing carriers of these genetic variants compared to wild-type subjects and looking at their blood pressure sensitivity to salt load or licorice exposure may help clarify the pathogenetic role 11 β HSD2 in essential hypertension.

8. Concluding remarks

The discovery that the juvenile hypertensive disorder called apparent mineralocorticoid excess has unmasked a new biological principle, in which hormone action in target organs is mediated by enzyme and not receptor specificity. Mutations or inhibition of 11 β HSD2 have clearly been shown to produce a congenital or acquired syndrome of mineralocorticoid excess, but both are altogether rare occurrences. Some circumstantial evidence points to a possible role of 11 β HSD2 as a mediator of increased blood pressure in humans. However, whether and to what extent subtle abnormalities in MR/11 β HSD2 mechanisms may contribute to essential hypertension is still unresolved.

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